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First- and second-generation immunometric PTH assays during treatment of hyperparathyroidism with cinacalcet HCl

KEVIN J. MARTIN, HARALD JÜPPNER, DONALD J. SHERRARD, WILLIAM G. GOODMAN, MARK R. KAPLAN, GEORGE NASSAR, PATRICIA CAMPBELL, MARIO CURZI, CHAIM CHARYTAN, LAURA C. MCCARY, MATTHEW D. GUO, STEWART A. TURNER, and DAVID A. BUSHINSKY

Saint Louis University, St. Louis, Missouri; Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts; Puget Sound Health Care System, Seattle, Washington; UCLA School of Medicine, Los Angeles, California; Nephrology Associates, Nashville, Tennessee; Medical Center Kidney Clinic, Houston, Texas; University of Alberta Hospital, Edmonton, Alberta, Canada; Diablo Clinical Research, Walnut Creek, California; New York Hospital Medical Center of Queens, Flushing, New York; Amgen, Inc., Thousand Oaks, California; University of Rochester, Rochester, New York

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Background. First-generation immunometric assays for "intact" parathyroid hormone (iPTH) also measure large N-terminally truncated PTH fragments, whereas second-generation assays, such as the "bio-intact" PTH (biPTH) assay, measure only full-length biologically active PTH(1–84). This study compared iPTH and biPTH assays during cinacalcet treatment in subjects with secondary HPT receiving dialysis.

Methods. Four hundred and ten subjects were enrolled in a 26-week randomized, double-blind, placebo-controlled trial of oral cinacalcet (or placebo), 30 to 180 mg once daily, and efficacy was assessed using biPTH and iPTH assays.

Results. Compared with control treatment, cinacalcet improved the management of secondary HPT. Both biPTH and iPTH decreased by 38% ± 3% during weeks 13 to 26 in the cinacalcet group; biPTH increased by 23% ± 4% and iPTH increased by 9.5% ± 3% in the control group ($P < 0.001$). Fifty-six percent of cinacalcet subjects and 10% of control subjects had a ≥30% reduction in biPTH, and 61% and 11%, respectively, had a ≥30% reduction in iPTH. Significant correlations between biPTH and iPTH levels were observed throughout the study. Both assays correlated similarly with bone-specific alkaline phosphatase levels. The ratio of biPTH to iPTH was maintained at 56% ± 1% after treatment in both treatment groups. Increasing serum calcium levels were associated with a decreasing ratio of biPTH to (iPTH–biPTH).

Conclusion. These data show that PTH can be monitored with either iPTH or biPTH assays during therapy with cinacalcet, and that cinacalcet therapy does not exert a major influence on the ratio between PTH(1–84) and large, N-terminally truncated PTH fragments.

Key words: bio-intact PTH, cinacalcet HCl, dialysis, immunometric PTH assay, intact PTH, parathyroid hormone, PTH fragments, secondary hyperparathyroidism.

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Measurements of parathyroid hormone (PTH) are essential for the diagnosis of secondary hyperparathyroidism (HPT) and for monitoring the patient's response to therapy. In addition, measurement of PTH helps avoid oversuppression of PTH secretion that contributes to adynamic bone disease [1–5], an increased risk of fractures in adults, and further impairment of longitudinal growth in children [6–8]. Measurement of PTH using the first-generation immunometric assay has been the most commonly used method over the past decade to diagnose and treat secondary HPT.

First-generation immunometric assays for PTH employ 2 antibodies; the detection antibody is directed toward an epitope within the N-terminal region of PTH, while the capture antibody is directed toward an epitope within the C-terminal region of PTH [1, 9]. Initially, first-generation immunometric assays were thought to detect exclusively full-length PTH(1–84), thus, the term "intact" PTH (iPTH) assay. Subsequently, it was found that such assays also detect large N-terminally truncated fragments, which behave on high-performance liquid chromatography (HPLC) similarly to PTH(7–84) [10, 11]. These large PTH fragments have not yet been defined chemically, but in patients with renal failure, they appear to be present in higher concentrations than in normal subjects [10–12]. Animal studies suggest this may be because of a somewhat reduced clearance of PTH fragments by the kidney and/or increased secretion of fragments from the parathyroid gland [13, 14].

Second-generation immunometric PTH assays were developed by different laboratories using detection antibodies that specifically recognize the first 1 or 2 N-terminal amino acids of PTH, and capture antibodies that recognize an epitope within the C-terminal region. The resulting sandwich assays are thus likely to exclusively detect the full-length, biologically active PTH molecule [i.e.,

PTH(1–84)] [15–17], unless PTH fragments truncated at the C-terminus are also present. Although theoretically, such second-generation PTH immunometric assays [e.g., Bio-Intact PTH Assay (Nichols Institute, San Clemente, CA, USA) (biPTH) [9], Whole PTH Assay (Scantibodies, San Clemente, CA, USA) [16], and Bioactive Intact PTH Assay (Immutopics, San Clemente, CA, USA)] [17], should be superior to the first-generation "intact" PTH assay, most current data suggest that PTH levels measured with either assay system provide a similar prediction of bone turnover in patients with end-stage renal disease (ESRD) [17].

Oral calcimimetic agents are small molecules that directly and rapidly lower PTH secretion by binding directly to the calcium-sensing receptor on chief cells in the parathyroid gland and increasing its sensitivity to extracellular ionized calcium [18]. Cinacalcet HCl (Sensipar®, Mimpara®, hereafter cinacalcet; Amgen, Inc., Thousand Oaks, CA, USA), the first calcimimetic agent to be evaluated in clinical trials for the management of secondary HPT [19–21], has recently been approved in the United States, Canada, and Europe for the treatment of secondary HPT in dialysis patients. The PTH values used in those studies to manage dose adjustments in cinacalcet therapy and to evaluate the efficacy of cinacalcet were measured using a first-generation PTH immunometric assay (iPTH Assay; Nichols Institute). The present analysis was conducted to determine whether second-generation PTH assays can be used to evaluate response to cinacalcet therapy with similar accuracy as first-generation assays, and to estimate the conversion factor from iPTH to biPTH levels during cinacalcet therapy. In addition, we sought to determine if demographic and laboratory parameters influence the ratio of biPTH to (iPTH–biPTH).

METHODS

Subjects

This prospective, multicenter, randomized, placebo-controlled trial was conducted at 65 centers in the United States and Canada. The primary inclusion criteria were age ≥18 years, thrice-weekly hemodialysis for at least 3 months, mean plasma iPTH level ≥300 pg/mL despite ongoing treatment with diet, vitamin D therapy, and/or phosphate-binding therapy, and mean serum calcium level ≥8.4 mg/dL. Enrollment of subjects with plasma iPTH >800 pg/mL at baseline was limited to 20% of the population. All subjects provided informed written consent to participate, and institutional review boards approved the study design.

Study design

Eligible subjects were randomly (computer-generated randomization) assigned in a 1:1 ratio to double-blind treatment with cinacalcet or matching placebo taken orally once daily; randomization was stratified by baseline iPTH and Ca × P level. Blood for biochemical measurements was drawn weekly from weeks 1 to 12 and then biweekly from weeks 13 to 26. Plasma PTH concentrations were measured by a first-generation assay, the Intact PTH Assay (iPTH) (Nichols Institute), which detects PTH(1–84) as well as PTH(7–84) [9, 15–17], and by a second-generation Bio-Intact PTH (biPTH) Assay (Nichols Institute), which detects PTH(1–84) [9]. The biPTH to iPTH ratio was determined by dividing the value obtained from the Bio-Intact PTH Assay by the value obtained from the Intact PTH Assay. The biPTH to (iPTH–biPTH) ratio was determined by dividing the value obtained from the Bio-Intact PTH Assay by the value obtained from the Intact PTH Assay minus the value obtained from the Bio-Intact PTH Assay. Serum bone-specific alkaline phosphatase (BALP) levels were determined before treatment and at weeks 12 and 26.

During the first 12 weeks, cinacalcet (or placebo) doses were titrated sequentially every 3 weeks from a starting dose of 30 mg to doses of 60, 90, 120, and 180 mg if plasma iPTH was >200 pg/mL and serum calcium was ≥7.8 mg/dL. The dose could be modified further as needed every 4 weeks during weeks 13 to 26. Dose reductions were permitted for symptomatic hypocalcemia, serum calcium <7.5 mg/dL, plasma iPTH <100 pg/mL on 3 consecutive study visits, or a dose-related adverse event.

Statistical analysis

Baseline values were obtained during the screening period. Mean values for iPTH and biPTH, calcium, phosphorus, and Ca × P were determined from all available results from weeks 13 to 26 (up to 7 values per subject). Evaluations included the proportion of subjects with an average iPTH ≤250 pg/mL and biPTH ≤140 pg/mL from weeks 13 to 26, and the proportion of subjects with an average iPTH and biPTH level from weeks 13 to 26 that was reduced by ≥30% from baseline. Subjects who withdrew before week 13 were included in the analysis and considered not to have met the study end points. The proportions of subjects who met the therapeutic end points were compared between groups by a Cochran-Mantel-Haenszel test, stratified by iPTH and Ca × P level at baseline.

Mean percentage changes in biPTH and iPTH from baseline were determined at each visit. The mean percentage changes from baseline during weeks 13 to 26 were compared between groups by a generalized Cochran-Mantel-Haenszel test using rank, stratified by iPTH and Ca × P values at baseline. The mean of the last 2 on-study values was carried forward for subjects who withdrew before week 13.

Correlation and linear regression analyses of plasma biPTH and iPTH values were performed for randomized subjects in each treatment group at baseline, as well as for

mean biPTH and iPTH values provided during weeks 13 to 26 to estimate the relationship between biPTH and iPTH values. Regression lines were fitted through the intercept of biPTH and iPTH. Subjects who withdrew from the study before week 13 were excluded from this analysis.

The linear regression analyses of biPTH to (iPTH–biPTH) were performed using mean values during weeks 13 to 26 or values at week 26, if appropriate. Data lines associated with negative (iPTH–biPTH) values (1.9% of the values) were removed before calculating means. Regression lines were fitted through the intercept of biPTH and (iPTH–biPTH) with treatment group and several demographic and laboratory variables as covariates. The difference in ratio of biPTH to (iPTH–biPTH) [i.e., coefficients of (iPTH-biPTH) in linear regression] between covariate subgroups and between treatment groups was tested for statistical significance using the contrast option within SAS procedure PROC GLM. The factors investigated were age, sex, race, coexistent diabetes, duration of dialysis, serum concentrations of calcium and phosphorus, BALP level, vitamin D use, and phosphate binder type used. All analyses were performed using SAS version 8.2 (Cary, NC).

RESULTS

Study participants

Baseline demographics and clinical characteristics of the study participants were not significantly different between treatment groups at baseline (Table 1). Mean ± SD age was 53.8 ± 14.4 years, 60% of subjects were male, 58% were black, 32% were white, and 10% were of other races. Participants had received dialysis treatment for an average of 64 months (range 0.5–290 months) before study entry. The mean ± SD plasma biPTH value at baseline was 332 ± 214 pg/mL (range 103–1712 pg/mL) and iPTH was 643 ± 370 pg/mL (range 299–2644 pg/mL). Mean ± SD serum calcium, phosphorus, and Ca × P values at baseline were 9.9 ± 0.78 mg/dL, 6.3 ± 1.7 mg/dL, and 61.6 ± 16.1 mg^2/dL^2, respectively.

At baseline, 69% of subjects were receiving vitamin D sterols. During the 6-month treatment period, 83% of subjects received at least 1 dose of vitamin D sterols and 98% received at least 1 dose of phosphate binders. Seventy-one percent of cinacalcet-treated subjects and 77% of control group subjects completed 26 weeks of treatment.

Response to treatment

Response to cinacalcet therapy was similar whether assessed using the biPTH or iPTH assay (Table 2). Mean concentrations of biPTH and iPTH during weeks 13 to 26 each decreased 38% ± 3% from baseline in cinacalcet-treated subjects ($P < 0.001$ vs. baseline). Control treatment during this same period was associated with mean increases in biPTH and iPTH of 23% ± 4% and 10% ± 3%, respectively ($P < 0.001$ vs. cinacalcet). Reductions in biPTH paralleled reductions in iPTH in the cinacalcet group throughout the study, with mean biPTH reductions ranging from 34% to 46% and mean iPTH reductions ranging from 37% to 46% at each visit from weeks 13 to 26 (Fig. 1). In the control group, mean biPTH increased by 19% to 24% from baseline during weeks 13 to 26 and mean iPTH increased by 6% to 13%. Cinacalcet therapy significantly reduced mean concentrations of serum calcium and phosphorus during weeks 13 to 26, compared with control ($P < 0.001$ and $P < 0.013$; Table 2). As would be expected, cinacalcet resulted in a significant ($P < 0.001$) reduction in Ca × P as well.

Cinacalcet reduced mean biPTH to ≤140 pg/mL in 45% of subjects, whereas only 8% of subjects in the control group ($P < 0.001$) achieved this reduction. Moreover, 39% of subjects in the cinacalcet group compared with only 3% of those in the control group experienced both a reduction in biPTH to ≤140 pg/mL and a reduction from baseline in Ca × P ($P < 0.001$). In addition, 56% of subjects in the cinacalcet group achieved a ≥30% reduction in biPTH from baseline, compared with 10% of subjects in the control group ($P < 0.001$). Similar results were observed when the iPTH assay was used to monitor response to therapy in the cinacalcet and control subjects [≤250 pg/mL (41% vs. 4%, $P < 0.001$), ≤250 pg/mL with Ca × P reduced (37% vs. 1%, $P < 0.001$), and ≥30% iPTH reduction (61% vs. 11%, $P < 0.001$)], respectively.

Table 1. Baseline demographics and clinical characteristics

	Cinacalcet ($N = 205$)	Control ($N = 205$)	P value[a]
Age *years* ± *SD*	53 ± 14	54 ± 15	0.504
Gender%			
Male/female	60/40	60/40	> 0.999
Race%			
White/black/other	30/59/11	34/58/8	0.666
Time on dialysis *months* ± *SD*	67 ± 56	62 ± 55	0.374
Vitamin D sterol use%	70	68	0.593
Calcitriol	8	9	
Paricalcitol	44	45	
Oral calcitriol	6	5	
Combined vitamin D therapy	<1	0	
Other	11	9	
Phosphate binder use%	94	95	0.661
Calcium-containing only	37	41	
Sevelamer only	38	36	
Calcium-containing/ sevelamer combination	15	13	
Other binders	4	5	

[a] P value determined using Student t tests for continuous variables or chi-squared tests for categorical variables.

Correlation between PTH assays and correlation with BALP

Regression analyses of plasma biPTH and iPTH values within each group resulted in highly significant

Table 2. Key efficacy results for intact parathyroid hormone (iPTH), bio-intact PTH (biPTH), calcium and phosphorus

	Cinacalcet ($N = 205$)		Control ($N = 205$)		
	Mean ± SD	Median (IQR)[a]	Mean ± SD	Median (IQR)[a]	*P* value[b]
Plasma iPTH					
Baseline[c]	636 ± 24	537 (399; 720)	646 ± 28	535 (393; 740)	0.711
Weeks 13-26[d]	385 ± 25[e]	275 (173; 448)[e]	698 ± 33	563 (378; 839)	<0.001
Percent change	−38.4 ± 2.9	−48 (−67; −19)	9.5 ± 2.8	4 (−15; 30)	
Plasma biPTH					
Baseline[c]	328 ± 14	266 (206; 363)	335 ± 16	276 (201; 366)	0.784
Weeks 13-26[d]	200 ± 15[e]	134 (78; 239)[e]	396 ± 18[e]	326 (207; 503)[e]	<0.001
Percent change	−38 ± 3	−49 (−70; −18)	23 ± 4	16 (−10; 46)	
Serum calcium					
Baseline[c]	9.8 ± 0.1	9.8 (9.3; 10.4)	9.9 ± 0.1	9.8 (9.4; 10.4)	0.888
Weeks 13-26[d]	9.2 ± 0.1[e]	9.1 (8.6; 9.6)[e]	9.9 ± 0.1	9.9 (9.4; 10.6)	< 0.001
Percent change	−6.3 ± 0.6	−5.5 (−13; −1)	0.5 ± 0.3	0.5 (−2; 4)	
Serum phosphorus					
Baseline[c]	6.3 ± 0.1	6.1 (5.3; 7.2)	6.2 ± 0.1	6.3 (5.1; 7.1)	0.331
Weeks 13-26[d]	5.7 ± 0.1[e]	5.6 (4.7; 6.5)[e]	6 ± 0.1	6.0 (5.2; 6.9)	0.013
Percent change	− 7.1 ± 1.7	−9 (−23; 5.9)	1.1 ± 1.8	−1 (−16; 12)	

[a]Interquartile range, reported as (25th, 75th) percentile of measured values.
[b]Between-group comparisons were performed using a generalized Cochran-Mantel-Haenszel test, stratified by baseline parathyroid hormone and dialysis modality.
[c]Based on 3 measurements during the screening period.
[d]The last 2 on-study values were carried forward for patients who withdrew during the titration phase.
[e]$P < 0.001$ vs. baseline, using a one-sample *t* test.

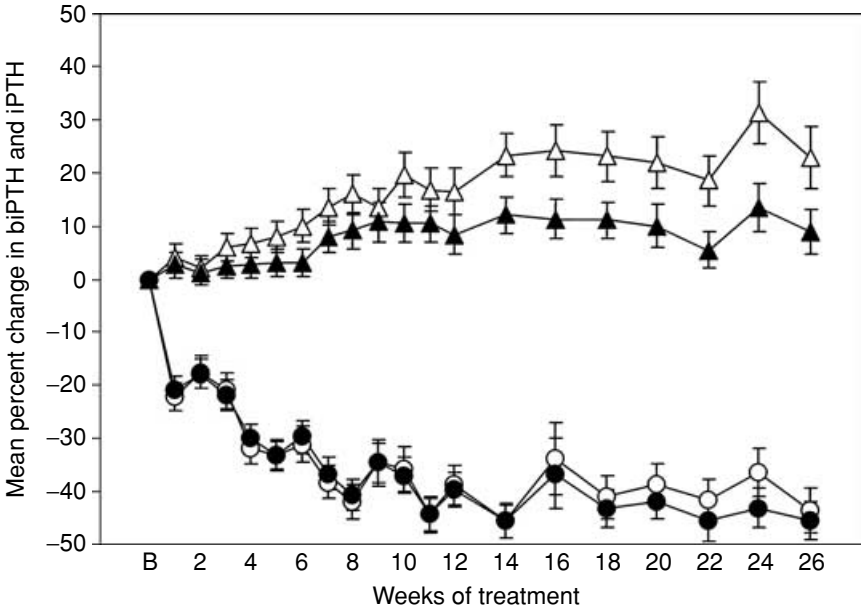

Fig. 1. Parathyroid hormone control by study treatment and study visit. Mean percent change from baseline in plasma intact parathyroid hormone (iPTH, closed symbols) and plasma bio-intact parathyroid hormone (biPTH, open symbols) during cinacalcet treatment (circles) and control treatment (triangles). B, baseline.

($P < 0.001$) correlations at baseline (cinacalcet $r = 0.877$, $P < 0.001$; control $r = 0.950$, $P < 0.001$). Similarly significant correlations ($P < 0.001$) were observed between mean plasma biPTH and iPTH values during weeks 13 to 26 of treatment (cinacalcet $r = 0.962$, $P < 0.001$; control $r = 0.949$, $P < 0.001$). The ratio of biPTH to iPTH was 52% ± 1% at baseline. During weeks 13 to 26, the ratio of biPTH to iPTH was 56% ± 1% in both the cinacalcet and control treatment groups. Both the biPTH and iPTH levels were significantly ($P < 0.001$) correlated with BALP levels at baseline, at the end of dose titration (week 12), and at the end of treatment (week 26) (Table 3), with the Pearson coefficients being higher for the cinacalcet group than the control group at both week 12 and week 26.

Influence of demographic and disease factors on the ratio of biPTH to (iPTH–biPTH)

The ratio of biPTH to (iPTH–biPTH) was 1.15 ± 0.04 during weeks 13 to 26 in the cinacalcet treatment group and 1.20 ± 0.03 in the control group ($P = 0.362$). Table 4 summarizes the analyses of demographic and clinical parameters that could have influenced the relationship between biPTH and (iPTH–biPTH) in either treatment

Table 3. Pearson correlation coefficients between bone-specific alkaline phosphatase (BALP) and intact parathyroid hormone (iPTH) or bio-intact parathyroid hormone (biPTH)

	Cinacalcet ($N = 205$)		Control ($N = 205$)	
	biPTH and BALP	iPTH and BALP	biPTH and BALP	iPTH and BALP
Baseline	0.55[a]	0.54[a]	0.51[a]	0.49[a]
Week 12	0.69[a]	0.61[a]	0.53[a]	0.58[a]
Week 26	0.73[a]	0.69[a]	0.42[a]	0.43[a]

[a] $P < 0.001$ for the correlation of biPTH and BALP or iPTH and BALP.

Table 4. Estimated mean (SE) ratio of biPTH/(iPTH-biPTH) by demographic and clinical characteristics[a]

	N	Cinacalcet	*N*	Control	*P* value[b]
Race					
White	52	1.09 (0.10)	57	1.11 (0.05)	0.82
Non-white	117	1.16 (0.05)	115	1.23 (0.03)	0.25
P value[c]		0.52		0.05	
Sex					
Male	106	1.11 (0.05)	105	1.20 (0.03)	0.13
Female	63	1.22 (0.07)	67	1.18 (0.05)	0.62
P value[c]		0.21		0.67	
Age					
<65 years	125	1.22 (0.05)	122	1.21 (0.03)	0.82
≥65 years	44	0.91 (0.09)	50	1.14 (0.06)	0.03
P value[c]		0.002		0.32	
Duration of dialysis					
0 to ≤2 years	43	0.94 (0.09)	45	1.16 (0.06)	0.04
2 to ≤5 years	54	1.31 (0.09)	67	1.16 (0.04)	0.14
>5 years	72	1.17 (0.06)	60	1.24 (0.04)	0.35
P value[c]		0.01		0.36	
Diabetes					
No	102	1.15 (0.05)	104	1.21 (0.03)	0.32
Yes	67	1.15 (0.08)	68	1.16 (0.05)	0.89
P value[c]		0.96		0.38	

[a]Estimates of ratios [i.e., coefficients of (iPTH-biPTH)] and statistical tests are from linear regression models by including biPTH as outcome and (iPTH–biPTH), treatment, 1 baseline characteristic, and their interactions as predictors.
[b]Across treatment group *P* value.
[c]Within treatment group *P* value for the effect of demographic or clinical characteristics.

group. The ratio between biPTH and (iPTH–biPTH) was somewhat higher in non-white patients in the cinacalcet group (1.09 vs. 1.16, for white and non-white respectively, $P = 0.52$) and this difference approached significance in the control group (1.11 vs. 1.23, $P = 0.05$). Men and women had a similar ratio of biPTH to (iPTH–biPTH) in the cinacalcet group (1.11 vs. 1.22, $P = 0.21$) and in the control group (1.20 vs. 1.18, $P = 0.67$). Subjects who were 65 years of age or older had a lower ratio of biPTH to (iPTH–biPTH) compared with younger subjects in the cinacalcet group (0.91 vs. 1.22, $P = 0.002$); the ratio was not significantly different by age in the control group (1.14 vs. 1.21, $P = 0.32$).

No clear relationship between the ratio of biPTH to (iPTH–biPTH) with increasing duration of dialysis at baseline was observed. Diabetes was not associated with a significant difference in the biPTH to (iPTH–biPTH) ratio in either treatment group.

Table 5. Estimated mean (SE) ratio of biPTH/(iPTH-biPTH) by on-study biochemical characteristics or concomitant medication use at weeks 13 to 26[a]

	N	Cinacalcet	*N*	Control	*P* value[b]
Calcium level					
≤ 9.5 mg/dL	121	1.23 (0.05)	52	1.31 (0.05)	0.30
> 9.5 mg/dL	48	0.97 (0.08)	120	1.16 (0.03)	0.02
P value[c]		0.004		0.01	
Phosphorus level					
≤ 5 mg/dL	57	1.00 (0.12)	35	1.14 (0.06)	0.29
> 5 to ≤ 6 mg/dL	51	1.02 (0.08)	54	1.16 (0.05)	0.14
> 6 mg/dL	61	1.25 (0.06)	83	1.23 (0.04)	0.74
P value[c]		0.03		0.40	
BALP					
≤ 15 ng/mL	64	1.00 (0.13)	43	1.20 (0.09)	0.21
> 15 to ≤ 25 ng/mL	51	1.01 (0.07)	39	1.17 (0.08)	0.14
> 25 ng/mL	39	1.27 (0.06)	76	1.22 (0.03)	0.40
P value[c]		0.01		0.83	
Vitamin D use at week 26[d]					
No	50	0.97 (0.09)	56	1.23 (0.04)	0.006
Yes	98	1.25 (0.05)	103	1.16 (0.04)	0.163
P value[c]		0.006		0.202	
Phosphate binder type used at week 26					
Sevelamer	46	1.17 (0.07)	64	1.25 (0.04)	0.39
Calcium-based	59	1.17 (0.08)	60	1.23 (0.05)	0.53
P value[c]		1		0.84	

[a]Estimates of ratios [i.e., coefficients of (iPTH-biPTH)] and statistical tests are from linear regression models by including biPTH as outcome, and (iPTH–biPTH), treatment, 1 of the biochemical characteristics, and their interactions as predictors.
[b]Across treatment group *P* value.
[c]Within treatment group *P* value for the effect of demographic or clinical characteristics, or laboratory values.
[d]Unadjusted for serum calcium level.

Influence of laboratory and concomitant therapy parameters on the ratio of biPTH to (iPTH–biPTH)

Table 5 summarizes the ratio of biPTH to (iPTH–biPTH) according to either laboratory values or concomitant therapy use at the same time PTH was measured (weeks 13 to 26 of the study). Higher serum calcium levels were associated with a lower ratio of biPTH to (iPTH–biPTH) in both treatment groups ($P = 0.004$ and 0.01 in the cinacalcet and control groups, respectively). In each serum calcium subgroup, the ratio of biPTH to (iPTH–biPTH) was lower in the cinacalcet group than in the control group. Higher serum phosphorus levels were associated with higher ratios of biPTH to (iPTH–biPTH) in the cinacalcet group ($P = 0.01$). A nonsignificant trend was observed in the control group. A higher level of BALP was associated with a higher ratio of biPTH to (iPTH–biPTH) in the cinacalcet group at week 26 ($P = 0.01$), but no relationship between BALP levels and this ratio was observed in the control group. A higher ratio of biPTH to (iPTH–biPTH) was observed in cinacalcet

subjects who received vitamin D sterols at week 26 ($P = 0.006$). When the vitamin D analyses were controlled for calcium levels, no significant effect of vitamin D use on the ratio was observed in the cinacalcet treatment group ($P = 0.919$ and 0.207 in the cinacalcet and control groups, respectively). Finally, no significant effect on the biPTH to (iPTH–biPTH) ratio of phosphate binder type used was observed.

Concomitant therapy use

There were no significant differences between treatment groups in the number of subjects who received vitamin D or phosphate binders at baseline and week 26. Vitamin D sterols were administered to 66% (136/205) and 65% (134/205) of subjects in the cinacalcet and control groups, respectively, at baseline. At week 26, vitamin D sterols were administered to 66% (98/148) of cinacalcet subjects and 65% (104/161) of control subjects. For phosphate binder use, 96% (196/205) and 95% (195/205) of cinacalcet and control subjects, respectively, received binders at baseline and 91% (135/148) and 98% (157/161), respectively, did so at week 26. The amount of elemental calcium ingested was similar between cinacalcet and control subjects both at baseline and at week 26. The mean ± SD for the elemental calcium dose (mg per main meal) was 677 (455) for cinacalcet subjects ($N = 87/200$) and 622 (334) ($N = 96/204$) for control subjects (P = NS) at baseline, and 741 (444) ($N = 45/200$) and 644 (350) ($N = 54/204$), respectively, (P = NS) at week 26.

DISCUSSION

This randomized, double-blind, placebo-controlled, multicenter trial in 410 subjects with secondary HPT on hemodialysis demonstrated that cinacalcet was effective in lowering PTH when either a first- or second-generation 2-site PTH immunometric assay was used to monitor response to treatment. Regression analyses revealed statistically significant correlations between assays at baseline and during weeks 13 to 26. The linear relationship between the 2 assays did not change after treatment with cinacalcet. Percentage reductions in biPTH and iPTH were 38% for each after treatment with cinacalcet. Approximately 60% of subjects treated with cinacalcet achieved a 30% or greater reduction from baseline in either iPTH or biPTH, whereas only 10% to 11% of subjects in the control group achieved this target. No significant differences between treatment groups in the percentage of subjects receiving vitamin D sterols or phosphate binders, or in the amount of elemental calcium ingested, were observed.

Several observational studies have reported statistically significant correlations between first- and second-generation immunometric PTH assays for healthy individuals and patients with primary HPT [22–24]. Similar correlations were reported in patients with ESRD, despite a wide range of PTH concentrations and different bone histomorphometric findings ranging from HPT to adynamic bone disease [25–28]. In this study, we report a strong correlation between PTH assays during prospective, double-blind treatment of dialysis patients with secondary HPT, indicating that results obtained using the biPTH assay can be used to monitor response to therapy with cinacalcet.

Because the correlation between plasma biPTH and iPTH in both treatment groups was excellent in the present study, a reasonable estimate of PTH(1–84) level could be derived by multiplying iPTH results by 0.54 (baseline ratio equaled 0.52 and the week 13 to 26 ratio equaled 0.56 for both treatment groups). This finding is consistent with previous trials suggesting that approximately 40% to 60% of total PTH detected by second-generation tests in patients with renal failure is actually PTH(1–84) [10], including a ratio of 64% in a retrospective study of hemodialysis patients that used the same commercial tests as this study [28]. Based on the findings of this study, the iPTH target of 150 to 300 pg/mL recommended by the NKF-K/DOQI™ guidelines [29] corresponds to an approximate biPTH target range of 80 to 160 pg/mL. Although bone histomorphometry was not assessed in this study**,** the consistent ratio between both assays observed by us and others [10, 15, 16, 28], and the similar correlations between biPTH or iPTH values and BALP, suggest that biPTH and iPTH have similar utility as predictors of bone health.

An advantage of this study was that the large number of subjects allowed for a meaningful analysis of the demographic and laboratory parameters that might have influenced the relationship between iPTH and (iPTH–biPTH). Overall, the ratio of biPTH to (iPTH–biPTH) was not significantly different between the cinacalcet and control treatment groups during weeks 13 to 26 (1.15 vs. 1.20, respectively). Slight modifications to the biPTH to (iPTH–biPTH) relationship may be observed based on demographic characteristics and/or laboratory parameters, and the trends observed in this study are discussed below.

Several previous studies in animals and humans suggested that intraglandular and peripheral metabolism of PTH to different fragments is regulated by blood ionized calcium concentrations [27, 30–34]. However, in these earlier studies, metabolism into fragments that resemble PTH(7–84) could not be assessed. When calcium levels are low, relatively more full-length PTH(1–84) is released from the parathyroid gland; whereas when calcium levels are high, relatively more PTH fragments are detected. Perhaps this regulation is designed to maximize the reabsorption of calcium in the distal tubule and

the mobilization of calcium from bone during hypocalcemia, and to prevent further elevations of serum calcium levels during hypercalcemia. In the current study, higher serum calcium levels were associated with a decreased ratio between biPTH and (iPTH–biPTH) in both the control and the cinacalcet groups; these results support earlier reports [30]. In addition, the ratio was lower in the cinacalcet group than the control group at both serum calcium levels examined, either because cinacalcet enhances the effect of calcium at the calcium-sensing receptor or because the distribution of calcium values within each serum calcium subgroup of cinacalcet-treated subjects is lower than in the subgroup of control subjects.

Elevated serum phosphorus levels stimulate PTH secretion [35]. A study by Chudek et al demonstrated that reductions in serum phosphorus levels with sevelamer treatment led to a decreased ratio of biPTH to iPTH [36], suggesting that more full-length PTH was produced when serum phosphorus levels were elevated. A significant increase in the biPTH to (iPTH–biPTH) ratio was observed for the highest serum phosphorus levels compared with the lowest serum phosphorus levels in the cinacalcet group; in the control group there was a trend toward a decrease in the ratio of biPTH to (iPTH–biPTH) with decreasing serum phosphorus levels. These results are consistent with the observation of Chudek et al, and are expected to maximize the renal excretion of phosphorus during hyperphosphatemia.

In general, demographic factors and other laboratory parameters did not have consistent effects on the ratio of biPTH to (iPTH–biPTH). In the cinacalcet group, subjects under 65 years old had a higher ratio of biPTH to (iPTH–biPTH) than subjects 65 years or older, but this effect was not observed in the control group. A trend for a higher biPTH to (iPTH–biPTH) ratio was observed for non-white subjects, compared with white subjects, but this only reached significance in the control group. The increased severity of secondary HPT observed in blacks, compared with whites, may contribute to this finding. A significantly higher ratio was observed in cinacalcet-treated subjects who had higher BALP levels, which is not unexpected because PTH and BALP are highly correlated, but this effect was not observed in the control group.

Initial analyses indicated that concurrent treatment with vitamin D sterols increased the ratio of biPTH to (iPTH–biPTH). Because many of the patients who enrolled in this study were not receiving vitamin D sterols due to hypercalcemia, these analyses were redone, adjusting for the serum calcium value. When the adjustment for serum calcium was included, there was no difference in the ratio by vitamin D use. Phosphate binder type used also did not alter the biPTH/(iPTH–biPTH) ratio.

CONCLUSION

Multiple studies have demonstrated the ability of cinacalcet therapy to reduce PTH levels as measured by the first-generation immunometric PTH assay [19–21, 37]. In this prospective, randomized, double-blind study of 410 subjects with secondary HPT receiving dialysis, we demonstrate that treatment response to cinacalcet therapy was nearly identical for iPTH and biPTH assays. The ratio of biPTH to iPTH (approximately 54%) was consistent throughout 6 months of cinacalcet or control treatment, across a wide range of iPTH values. The biPTH to (iPTH–biPTH) ratio was also consistent (approximately 1.2). Statistically significant variations in the ratio of biPTH to (iPTH–biPTH) were observed with variations in serum calcium. In conclusion, for hemodialysis patients with secondary HPT, the newer second-generation PTH assays appear to have comparable accuracy for monitoring PTH response to cinacalcet therapy compared with first-generation assays.

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Reprint requests to Kevin J. Martin, M.B., BCh, FACP, Division of Nephrology, Saint Louis University, 3635 Vista Avenue, St. Louis, MO 63110.
E-mail: martinkj@slu.edu

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